



King's Research Portal

DOI:

[10.1016/j.jid.2016.08.026](https://doi.org/10.1016/j.jid.2016.08.026)

Document Version

Peer reviewed version

[Link to publication record in King's Research Portal](#)

Citation for published version (APA):

Kaushal, G., Rognoni, E., Lichtenberger, B. M., Driskell, R. R., Kretzschmar, K., Hoste, E., & Watt, F. M. (2017). Letter in reply to Chi et al. *Journal of Investigative Dermatology*, 137(1), 247-248.
<https://doi.org/10.1016/j.jid.2016.08.026>

Citing this paper

Please note that where the full-text provided on King's Research Portal is the Author Accepted Manuscript or Post-Print version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version for pagination, volume/issue, and date of publication details. And where the final published version is provided on the Research Portal, if citing you are again advised to check the publisher's website for any subsequent corrections.

General rights

Copyright and moral rights for the publications made accessible in the Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognize and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the Research Portal

Take down policy

If you believe that this document breaches copyright please contact librarypure@kcl.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

Accepted Manuscript

Letter in Response to “Stabilization of β -catenin does not increase dermal papilla cell number in the hair follicle”

Grace Kaushal, Emanuel Rognoni, Beate M. Lichtenberger, Ryan R. Driskell, Kai Kretzschmar, Esther Hoste, Fiona M. Watt

PII: S0022-202X(16)32370-3

DOI: [10.1016/j.jid.2016.08.026](https://doi.org/10.1016/j.jid.2016.08.026)

Reference: JID 517

To appear in: *The Journal of Investigative Dermatology*

Received Date: 11 August 2016

Accepted Date: 16 August 2016

Please cite this article as: Kaushal G, Rognoni E, Lichtenberger BM, Driskell RR, Kretzschmar K, Hoste E, Watt FM, Letter in Response to “Stabilization of β -catenin does not increase dermal papilla cell number in the hair follicle”, *The Journal of Investigative Dermatology* (2016), doi: 10.1016/j.jid.2016.08.026.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



Dr Barbara Gilchrest
 Editor in Chief
 Journal of Investigative Dermatology

11th August 2016

Dear Dr Gilchrest,

JID-2016-0673 - Stabilization of β -catenin does not increase dermal papilla cell number in the hair follicle

Thank you for your e-mail of 4th August, providing us with the opportunity to respond to the conclusions of this paper. First of all we are grateful to Dr Morgan and his colleagues for engaging in a dialogue over our own paper (Kaushal et al., 2015), which began when he was one of the reviewers. We believe that open discussion about conflicting observations is important in moving forward research – far too often results that are at odds with one another are simply ignored.

There are certainly aspects of the Chi et al study that are superior to ours. In particular, we agree that there are advantages of studying the DP in unpigmented skin and we note that the quantitation by Chi et al is based on a larger number of hair follicles, and mice, than in our study.

Chi et al suggest that our results are due to misidentification of hair follicle types. We agree that DP size alone cannot be used to identify hair follicle type. We analysed skin of P65 mice in which the HF cycle was asynchronous, so that we could report on anagen (stage IV; early anagen of the second hair follicle cycle, according to the classification of Muller-Rover et al., 2001) and telogen follicles from the same mice. We believe that Chi et al are looking at a later anagen stage. Given the low frequency of non-zigzag hairs in our samples it is unlikely that they would skew our data.

The tissue in the Kaushal et al paper was not "flash frozen" but fixed with 4% paraformaldehyde before being cryopreserved in OCT medium. Tissue preservation under these conditions is excellent (Driskell et al., 2012 & 2013). The movies in the Supplemental Material of Kaushal et al show that the 60um thick horizontal wholemounts are sufficient to capture all of the cells within a DP.

Several differences in the experimental approaches could contribute to the different results:

1. Chi et al use Cre, not CreER, for genetic labelling and thus do not obtain temporal control of Cre activity. Further no control for efficient recombination / β catenin stabilisation using this Cre line is provided in the current or previous (Enshell-Seijffers et al., 2010) study.
2. In the 2010 Dev Cell paper by Enshell-Seijffers et al., the authors describe Corin-Cre activity as being first detected at P3 in some DP cells; however, Corin is not homogenously expressed in all DP cells until P7. In contrast, Kaushal et al treated with Tamoxifen to induce

CreER at P1 and P2, before Corin-Cre is active. The use of different promoters to drive Cre expression and the activation of Cre at different times prevents direct comparison of the results.

3. Having worked with a number of different reporter lines, in our opinion the Rosa26-tdTomato reporter mouse line of Kaushal et al is superior to the Rosa26-YFP reporter used by Chi et al, both in terms of recombination sensitivity and fluorophore expression level.

Finally, two recent papers by Zhou et al support the conclusions of Kaushal et al. These authors found that 'expression of ΔN - β -catenin in CD133+ DP cells leads to increased DP cell proliferation' and 'in line with this finding, the number of DP cells was increased in mutant hair follicles... Analysis of skin histology showed that the mean size of DPs in mutant CD133-CreERT2; Rosa-rtTA; tetO-Ctnnb1 ΔN hair follicles ... was increased compared with controls during early anagen stages..' (Zhou et al., 2016a). These authors have further reported that expression of a stabilized form of β -catenin promotes clonal growth of CD133-positive (CD133+) DP cells in an in vitro three-dimensional hydrogel culture and on transplantation promoted in vivo hair growth in reconstituted skin compared to control cells (Zhou et al., 2016b).

In closing, we wish to thank you once again for the opportunity to discuss our results and look forward to further important contributions from the Morgan lab on the regulation and function of the dermal papilla.

Yours sincerely,

Grace Kaushal
Emanuel Rognoni
Beate M. Lichtenberger
Ryan R. Driskell
Kai Kretzschmar
Esther Hoste
Fiona M. Watt*

*Corresponding author: Centre for Stem Cells and Regenerative Medicine, King's College London, 28th Floor, Tower Wing, Guy's Hospital, Great Maze Pond, London SE1 9RT, UK
fiona.watt@kcl.ac.uk

References

Driskell RR, Juneja VR, Connelly JT, Kretzschmar K, Tan DW, Watt FM. (2012) Clonal growth of dermal papilla cells in hydrogels reveals intrinsic differences between Sox2-positive and -negative cells in vitro and in vivo. *J Invest Dermatol.* 132:1084-93.

Driskell RR, Lichtenberger BM, Hoste E, Kretzschmar K, Simons BD, Charalambous M, et al. (2013) Distinct fibroblast lineages determine dermal architecture in skin development and repair. *Nature.* 504:277-81.

Enshell-Seijffers D, Lindon C, Kashiwagi M, Morgan BA. (2010) beta-catenin activity in the dermal papilla regulates morphogenesis and regeneration of hair. *Dev Cell*. 18:633-42.

Kaushal GS, Rognoni E, Lichtenberger BM, Driskell RR, Kretschmar K, Hoste E, *et al.* (2015) Fate of prominin-1 expressing dermal papilla cells during homeostasis, wound healing and Wnt activation. *J Invest Dermatol*. 135:2926-2934.

Müller-Röver S, Handjiski B, van der Veen C, Eichmüller S, Foitzik K, McKay IA, *et al.* (2001). A comprehensive guide for the accurate classification of murine hair follicles in distinct hair cycle stages. *J Invest Dermatol*. 117:3-15.

Zhou L, Xu M, Yang Y, Yang K, Wickett RR, Andl T, *et al* (2016a) Activation of β -catenin signaling in CD133-positive dermal papilla cells drives postnatal hair growth. *PLoS One*. 11:e0160425.

Zhou L, Yang K, Xu M, Andl T, Millar SE, Boyce S, *et al.* (2016b). Activating β -catenin signaling in CD133-positive dermal papilla cells increases hair inductivity. *FEBS J*. 283:2823-35.